

CHAPTER 18

EPHRUSSI/BEADLE/TATUM: GENES ENCODE ENZYMES

George Beadle and Boris Ephrussi did pioneer work on Drosophila eye transplants in 1935 to study the effect of host enzymes on transplanted tissue. In 1940, the work was expanded by Beadle and Edward Tatum on thiamine requirements in the bread mold Neurospora, leading them to propose their “one gene-one enzyme” theory.

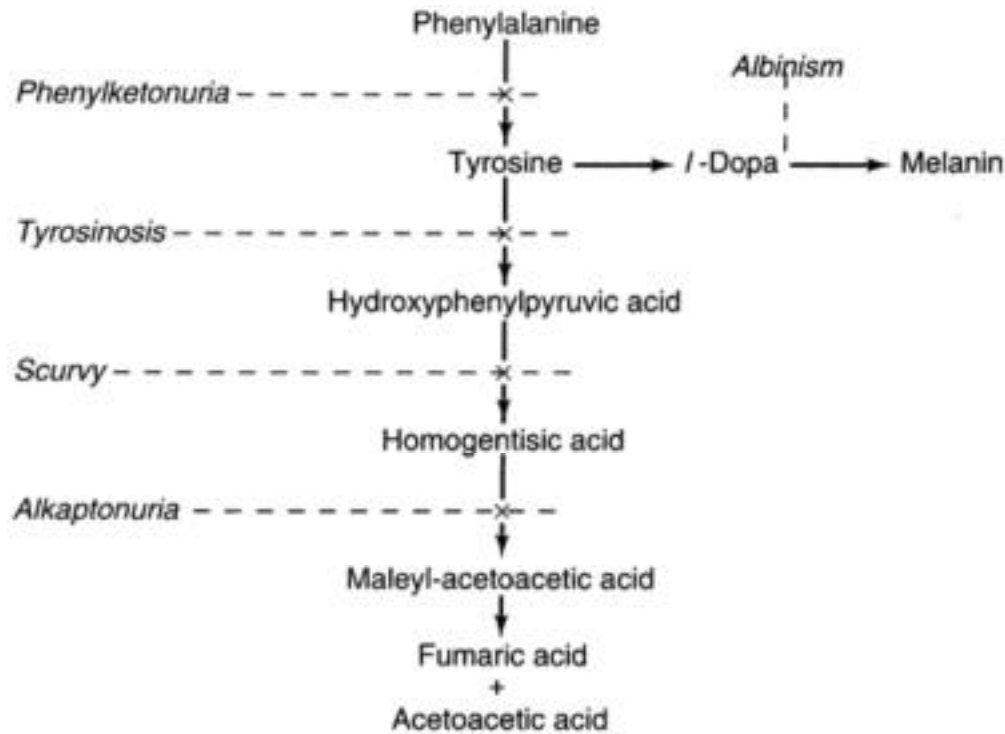
GARROD’S “INBORN ERRORS OF METABOLISM”

The first clear recognition that novel phenotypes may reflect discrete biochemical differences was provided by the English physician Archibald E. Garrod at the turn of the 20th century. In 1902, barely after the rediscovery of Mendel’s work, Garrod described a disease, *alkaptonuria*, in which affected patients produced urine that turned black upon exposure to air—a rather disconcerting symptom. The blackening proved to be due to the oxidation of *homogentisic acid* (alkapton) in the urine of affected patients. Normally, homogentisic acid is broken down in the liver and is not present in the urine. Garrod concluded that alkaptonuric patients lack the liver enzyme (homogentisic acid oxidase) necessary to metabolize homogentisic acid. Unable to process homogentisic acid, the patients accumulate it and excrete it in their urine.

Garrod’s key observation was that alkaptonuria was a hereditary condition. When one family member had it, others tended to also; children of first cousins exhibit it more often than those of unrelated people. If the loss of a particular liver enzyme is a heritable trait specified by a particular gene allele, then it follows that the presence of an active form of that enzyme is also specified by an alternative allele of that gene. The presence or absence of the alkaptonuric phenotype depends on the absence or presence of a workable copy of the gene-encoded enzyme.

Garrod’s discovery, ignored for 30 years, provided the experimental key to dissecting metabolically determined phenotypes. Alkaptonuria (the lack of homogentisic acid oxidase activity) is *detected* by the buildup of the substrate of the missing enzyme (homogentisic acid). In principle, the metabolic role of any enzyme contributing to a phenotype can be determined in this manner by examining mutant individuals to ascertain which compound they accumulate. Experimental problems of isolation and chemical identification are significant, but the general approach is clear.

A variety of human diseases are now known to reflect simple enzyme deficiencies. Some very famous one prove to alter steps on the same biochemical pathway as alkaptonuria:



EPHRUSSI AND BEADLE'S EXPERIMENT ON DROSOPHILA

The first geneticists to extend Garrod's seminal observation were Boris Ephrussi and George Beadle in 1935, studying *Drosophila* eye color mutants. They set out to see if they could dissect the "eye color" phenotype into discrete genetic components. They first isolated 26 different eye color mutants, each heritable and with a distinctive phenotype. They then devised an ingenious experimental approach: they transplanted the larval embryonic eye tissue from each mutant into the abdominal area of a wild-type larva, allowed the host larva to develop into an adult, and then ascertained the color of the vestigial abdominal eye.

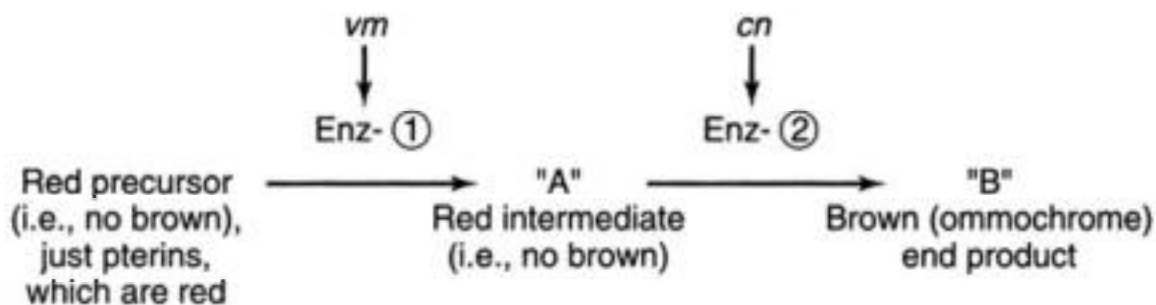
In almost all cases, the mutant eye tissue was not affected by transplantation; transplanted eyes develop the same color as the stock from which they had come. This result served to verify the genic nature of the mutant eye color phenotypes. It was not the larval tissue *environment* that determined color, but the larval *genes*.

However, of the 26 different eye color mutant that Ephrussi and Beadle examined, two gave quite a different result. Vermilion (*vm*) and cinnabar (*cn*), both with bright red rather than wild-type brown eyes, developed wild-type eye color upon tissue transplantation into wild-type larvae! Some diffusible substance was penetrating into the *vm* and the *cn* larval tissue from the surrounding wild-type tissue, a substance which permitted full pigment development in the mutant tissue.

ANALYSIS OF METABOLIC PATHWAYS

Could it have been that the enzyme missing in *vm* and *cn* flies was diffusing in from the wild-type tissue? No. Proteins are too big to readily diffuse from cell to cell. Presumably what was being supplied was a metabolite, perhaps the product usually produced by the missing enzyme activity.

Was the substance the same for *vm* and *cn*? This was the crux of the matter, the point Ephrussi and Beadle had originally set out to test. Did *vm* and *cn* make the same contribution to eye color phenotypes? The test was straightforward and rigorous: if the same substance converted both *vm* and *cn* tissue phenotypes to wild-type, then a transplant of *vm* into *cn* larvae or a transplant of *cn* into *vm* larvae should never result in the conversion of the transplanted tissue phenotype to wild-type. If *vm* and *cn* were deficient in the same metabolic step, then one could not give the other what it itself lacked. Were *vm* and *cn* the same? No. When the transplant went from *cn* → *vm*, the eye tissue phenotype remained mutant bright red, but when the transplant was from *vm* → *cn*, the eye tissue phenotype was wild-type! Thus, *vm* larval tissue was unable to supply the *cn* transplant with a metabolite past the *cn* blockage; *vm* tissue must not have been using this part of the pathway. *Cn* larval tissue could supply the *vm* transplant with a metabolite past the *vm* blockage; *cn* tissue had to be utilizing the *vm* part of the metabolic pathway leading to wild-type eye color. Thus, *vm* and *cn* represented distinctly different steps in the metabolic process determining *Drosophila* eye color. They altered different steps in the same process or pathway, and the order of their activities was *vm* and then *cn*. The *vm* and *cn* phenotypes resulted from the mutational loss of two different enzyme activities:



cn flies can supply "A" to *vm* flies
 (Reaction ① still works, even though
 Reaction ② is blocked.)

vm flies cannot supply "B" to *cn* flies!
 (Reaction ② works, but there is no substance for it
 to work on, because Reaction ① doesn't work.)

EPISTASIS AND OTHER OBSTACLES

These experiments served to point out the difficulty of analyzing complex phenotypes when many genes contribute to the final realized state. A fly that was homozygous for *vm* did not reveal the state of the *cn* gene—mutant or wild-type—it looked the same, as the *cn* gene's product acted at a position on the pathway after the *vm* blockage. The ability of one mutation to mask the effect of another, preventing its detection, is called *epistasis*.

A second difficulty involved the task of chemically identifying the substances accumulated behind various enzyme blockages. While this could be done, considerable effort was required. A far simpler approach was not to identify the accumulated substance, but rather to identify the substance immediately past the blockage: what could be supplied to the tissue that would let the pathway proceed? Because the identity of what was being supplied was known, a trial-and-error screening of possibilities would pinpoint the correct metabolite. It was difficult to supplement *Drosophila* tissue in a controlled way, so to pursue this problem further, Beadle transferred his attention to a simpler eukaryote, the bread mold *Neurospora crassa*. That work was carried out in collaboration with Edward Tatum, and was awarded the Nobel Prize in 1958.

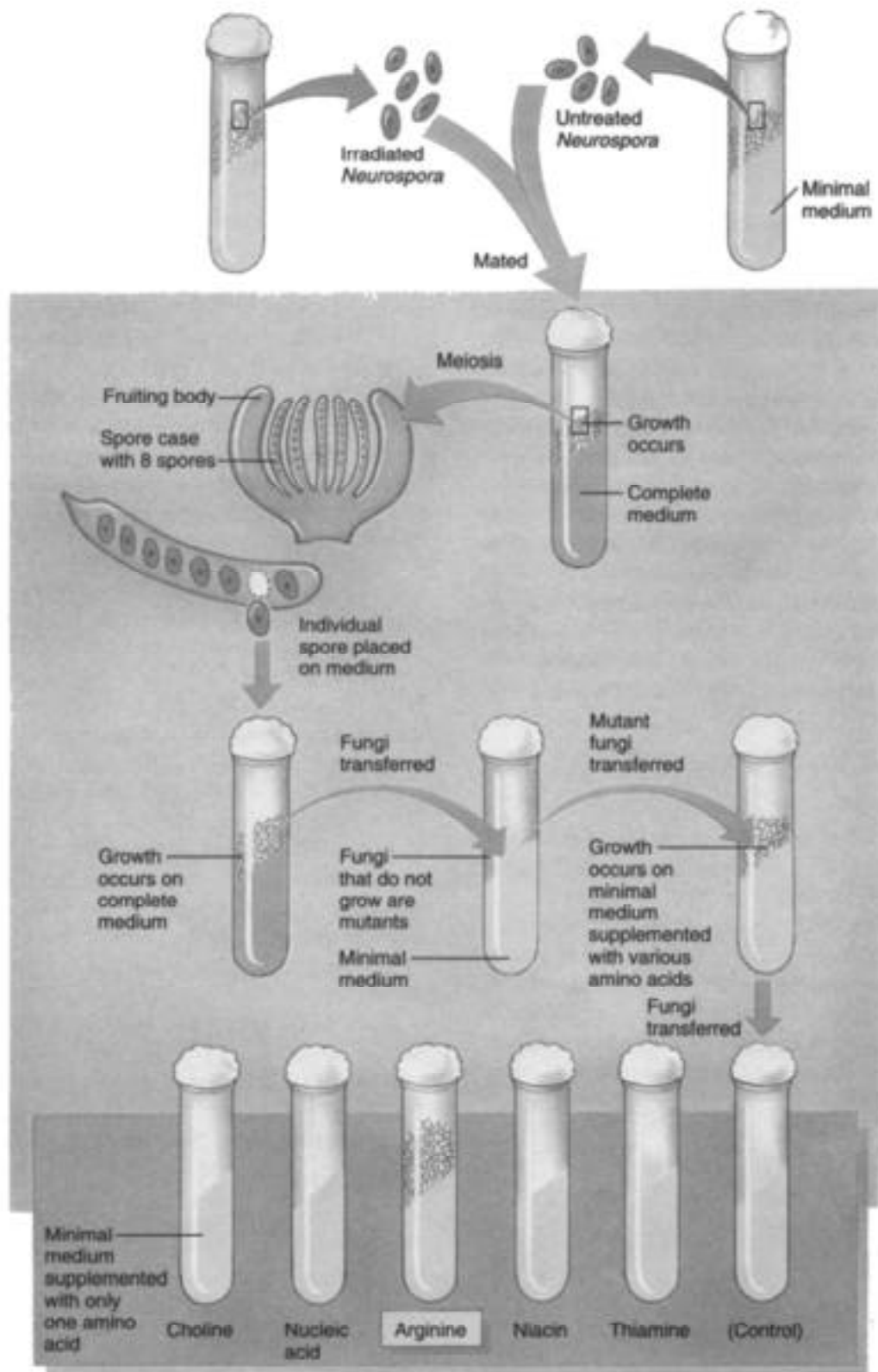
BEADLE AND TATUM'S EXPERIMENT ON NEUROSPORA

Instead of transplanting two phenotypically similar eye color mutants to see if they involved the same or different genes, Beadle and Edward Tatum supplemented two phenotypically similar *auxotrophic microbes* (microbes unable to synthesize certain materials, which subsequently needed to be supplied in the growth medium) with different compounds in the affected pathway: mutants in different steps responded to different supplements. Thus, when four thiamine-requiring *Neurospora* mutants were tested for their ability to grow when supplemented with intermediates in the thiamine biosynthetic pathway, four different results were obtained:

1. *thi-2* would grow if supplemented by any of the pathway intermediates. This mutation blocked the initial step of the pathway, so that all intermediate compounds occurred past the *thi-2* block.
2. *thi-4* would not grow if supplemented with pyrimidine, as the *thi-4* block occurred after that step. It would grow if supplemented by either thiamine or its phosphorylated immediate precursor, so the *thi-4* block must have occurred prior to the synthesis of these two compounds.
3. *thi-3* would grow only when supplemented with thiamine. The *thi-3* block must have occurred immediately prior to the synthesis of thiamine.
4. *thi-1* would grow if supplemented with any of the pathway intermediates, just as would *thi-2*.

How could one determine that *thi-1* and *thi-2* were not two isolates of the same mutation? A complementation test was performed, not different in principle from the *vm - cn* transplantation test of Beadle and Ephrussi. Hyphae strands of *thi-1* and *thi-2* were allowed to grow in contact with one another. In contact, cell fusion could occur, producing a *heterokaryon*, a hybrid cell containing both sets of nuclei in a common cytoplasm. If *thi-1* and *thi-2* were mutations in *different* genes, then the hybrid heterokaryon line would be able to grow on minimal medium, because it possessed at least one good copy of both genes. If *thi-1* and *thi-2* were mutations in the *same* gene, then the heterokaryon line would *not* grow on minimal medium because it had no good copy of the mutant gene in the thiamine pathway and thus could not make its own thiamine.

This same procedure may be used to dissect most simple biochemical pathways into their component parts (figure 18.1). The results of supplementation are usually arrayed in a simple table, deducing the order of the steps in the pathway from the pattern of growth.



(a)

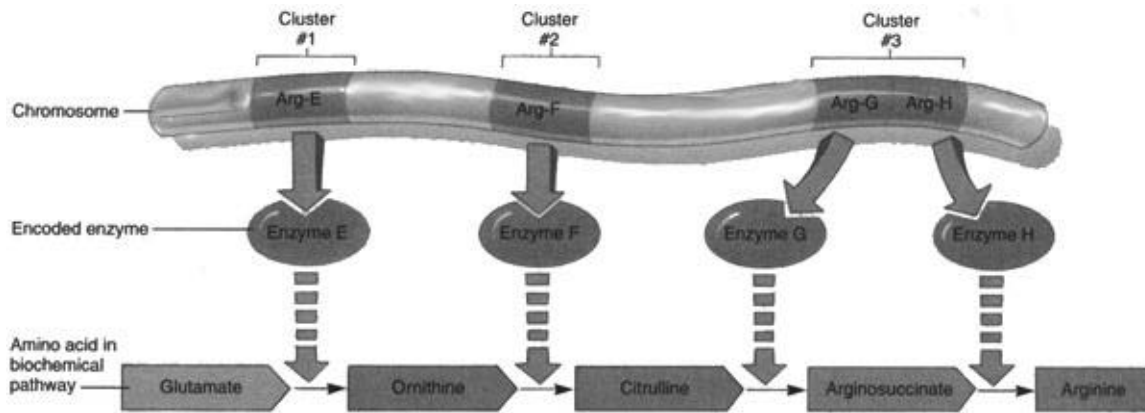


Figure 18.1

(a) The Beadle-Tatum experiment: isolating nutritional mutations in Neurospora. (b) Evidence for the “one gene-one enzyme” hypothesis. The chromosomal locations of the many arginine mutations isolated by Beadle and Tatum cluster around three locations, corresponding to the locations of the genes encoding the enzymes that carry out arginine biosynthesis.